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EXPERIMENTS ON THE ISOLATED MAMMALIAN HEART TO SHOW THE RELATION OF THE INORGANIC SALTS TO THE ACTION OF THE ACCELERATOR AND INHIBITORY NERVES.

By W. H. HOWELL and W. W. DUKE[1].

(*From the Physiological Laboratory of the Johns Hopkins University.*)

In previous papers one of us[2] has suggested the possibility that "the inhibitory influence of the vagus and the augmenting influence of the sympathetic upon the heart may be exerted through some intermediate effect upon the potassium and calcium compounds in the heart tissue." Experiments made upon the cold blooded heart with reference to the effect of potassium salts upon vagus inhibition seemed to support this view, and the following series of experiments were therefore made upon the mammalian heart with especial reference to the relations of the calcium salts to the action of the accelerator nerves. Hering[3] has shown that the isolated mammalian heart, kept beating by a circulation of Ringer's liquid through the coronary vessels, continues to show inhibition and acceleration when the corresponding nerves are stimulated. The accelerator nerve especially maintains its action for many hours. In Hering's experiments the animal was bled first, and afterward the nerves were isolated and the other operations necessary for the artificial circulation were performed. Our first attempts followed this procedure, but were for the most part unfortunate, since by the time that the circulation was established the heart exhibited fibrillary contractions. From this condition the heart could be resuscitated usually by Hering's method of first bringing it to complete standstill by injecting a strong solution of potassium chloride (1 p.c.), and then

[1] During a portion of this investigation the authors were assisted by Mr J. E. Burns, junior, to whom they desire to express their thanks.

[2] W. H. Howell. *Amer. Journ. of Physiol.* VI. pp. 181—206. 1901. *Ibid.* XV. pp. 280—294. 1906.

[3] H. E. Hering. *Arch. f. d. ges. Physiol.* XCIX. pp. 245—252. 1903.

circulating the Ringer's liquid. We found it more advantageous, however, to so perform the operation as to keep the heart beating until the Ringer's solution was turned on. In this way the heart continued to beat without interruption and fibrillary contractions did not appear. Our method was as follows. The animals used were cats, since it was found that the heart of this animal beats more certainly on the artificial circulation than that of dogs. We had in fact no failures with the heart of this animal. The cats were anæsthetized with ether. The upper portion of the sternum was resected, the internal mammary arteries were ligated, artificial respiration was established and the chest was then widely opened on both sides. The brachio-cephalic artery was clamped close to the aorta and the inflow cannula for the artificial circulation was inserted. The left subclavian was then ligated at the level of the annulus of Vieussen and a loose ligature was laid round the aorta just beyond the origin of the left subclavian. The aorta was opened beyond this ligature, and the artificial circulation was turned on through the brachio-cephalic. After running for a few minutes, to wash out the blood, the aortic ligature was tied and the inferior vena cava was opened widely near the auricle. The heart was then beating upon the Ringer's mixture, and while this was circulating the accelerator nerve was prepared for stimulation, the pericardium was opened freely, and levers were attached to the left auricle and the right ventricle to record their movements. The accelerator nerve of the left side was used in all cases. Most observers state that the accelerator of the right side is more effective, but for our purpose the nerve on the left side was more convenient and it was found to give in all cases very marked acceleration and augmentation. As described by Boehm[1] the large stellate ganglion in the cat gives off the two nerves forming the annulus, and close to the origin of the one which runs ventral to the subclavian sends off also a large branch, the nervus accelerans, or nervus cardiacus e ganglio stellato, toward the heart. A third smaller branch arises in some cases from the ganglion close to the two mentioned and runs toward the heart along the subclavian artery. These three branches, 1, 2 and 3 in the accompanying sketch (Fig. 1), were usually stimulated together, since each of them might contain accelerator or augmentor fibres and they arise so near together that it is convenient to lift them on the electrodes in a bunch. In some cases branch 3, the nervus accelerans proper, when it happened to be well developed, was stimulated alone. The individual animals differed somewhat in respect to the size of this

[1] R. Boehm. *Arch. f. exp. Pathol. u. Pharmakol.* IV. p. 255. 1875.

branch. To record the movements of the heart the pericardium was first opened freely and the apex of the heart was then anchored by passing a hook through it and tying the ligature from the hook to some fixed point. Hooks were then inserted into the tip of the left auricular appendage and into the lateral surface of the right ventricle. Through ligatures attached to these hooks the movements of these chambers were communicated to receiving tambours conveniently placed, and thence by connecting tubing to recording tambours which wrote upon the blackened paper of a Hürthle kymographion. A time pen and a stimulating pen recorded on the same surface.

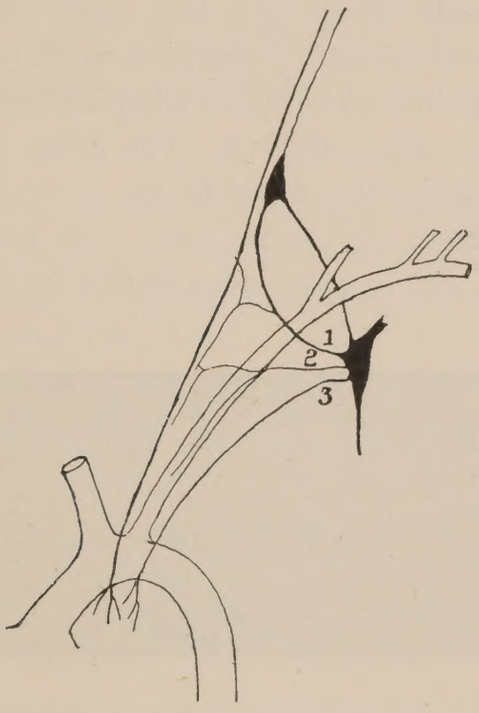

Fig. 1. Sketch to show the accelerator (and augmentor) branches from the stellate ganglion (in the cat) which were stimulated in these experiments.

1, the ventral branch of the annulus. 2, small branch not constantly present. 3, Boehm's accelerator nerve (n. cardiacus e ganglio stellato).

To maintain the beats of the heart Locke's modification of Ringer's solution was employed. This mixture contained sodium choloride 0·9 p.c., calcium chloride 0·023 p.c., potassium chloride 0·025 p.c., sodium bicarbonate 0·02 p.c., and dextrose 0·10 p.c. The solution was fed from large bottles arranged as Mariotte's flasks to maintain a constant pressure, and the air tube of these flasks was connected with a large gasometer containing oxygen, so that the circulating medium was kept constantly under a pressure of one atmosphere of oxygen. The liquid was supplied to the coronary artery at a low temperature, 30° to 35° C., and at

a comparatively low pressure of 60 to 65 mms. of mercury. Under these conditions the heart beat very well indeed for several hours; stimulation of the vagus nerve gave typical inhibition at the beginning of the experiment, but usually ceased to have an effect after the first hour or so. Stimulation of the accelerators on the contrary gave excellent results throughout the four or five hours for which the experiments were continued, unless the heart was injured by extreme variations of the composition of the circulating liquid.

Action of the accelerator nerve when the heart was fed with the standard Ringer's mixture. As stated above all stimulations of the accelerators were made upon the left side. When the heart was running on the standard mixture containing 0·023 p.c. of calcium chloride and 0·025 p.c. of potassium chloride an adequate stimulus gave a marked acceleration as well as augmentation. The latter feature was especially marked. An increase in the force of the beat was always the first indication of an effect. In some cases the augmentation occurred without acceleration, and in some cases reduction in the strength of the

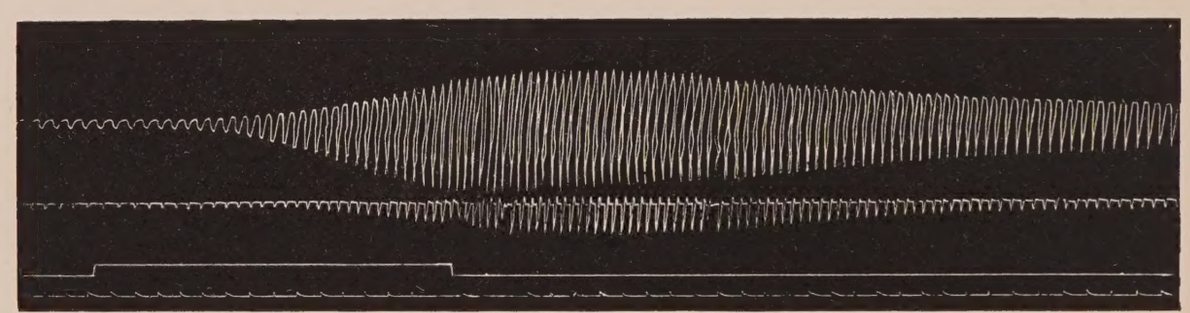

Fig. 2. To show the acceleration and augmentation produced by a strong stimulus (100). The upper curve gives the ventricular contractions, the lower one the auricular contractions. The lowermost line gives the time in seconds, and the line above indicates the duration of the stimulation of the accelerator nerve.

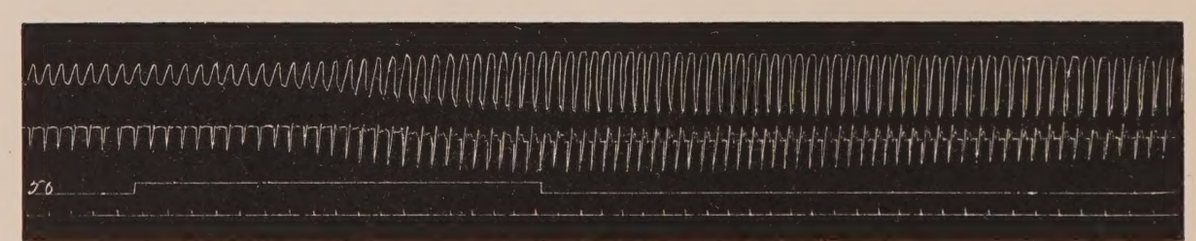

Fig. 3. To show the acceleration and augmentation produced by a moderate stimulus (50). The upper curve gives the ventricular contractions, the lower one the auricular contractions. The time in seconds and the duration of the stimulus are indicated in the two lower lines.

stimulus was followed by a disappearance of the acceleration while the augmentation still remained. Figures 2 and 3 give typical examples of the usual effect obtained, and the following data illustrate the fact that the augmentation may occur independently of the acceleration.

	Stimulus[1]	Acceleration	Augmentation (Ventricle)
Exp. 18.	50	None (102)	11 to 16 mm.
	50	None (111)	7½ to 16½ mm.
	50	None (105)	9 to 13 mm.
Exp. 11.	150	81 to 129	20 to 30 mm.
	80	81 to 102	14 to 24 mm.
	70	None (81)	14 to 16 mm.
Exp. 15.	50	94·5 to 105	4 to 12·5 mm.
	20	None (87)	5 to 11 mm.

[1] The strength of the stimulus is expressed in current units as given upon a Petzold induction coil.

So far as the accelerators of the left side are concerned, augmentation rather than acceleration was the predominating effect upon the heart. In some cases when the stimulus was strong the augmentation in force of the beat was remarkable, both auricles and ventricles contracting with a vigour which was in marked contrast to their normal beats. The tambour records did not always transcribe faithfully the full effect of this increase. The fact that in certain hearts augmentation alone was obtained, and in others the same result followed upon a diminution in the strength of the stimulus or in the concentration of the calcium in the circulating liquid, indicates strongly the correctness of the view that these two effects depend upon the activity of separate fibres. The augmentor fibres respond to weaker stimuli, or at least the augmentor effect comes out more easily than that of acceleration, just as in the action of the vagus fibres a weakening of the beat may be obtained with minimal stimuli without any effect upon the rate. In only one of the experiments performed was there any indication of a reverse action, a condition namely in which the nerve gave acceleration without augmentation. In this experiment the vagus had been stimulated above the arch of the aorta until it ceased to give a visible response. The vagus together with some of the neighbouring accelerator branches which run from the ganglion to the arch of the aorta was then stimulated. The result was a marked acceleration, while the force was diminished rather than increased. As this effect was never obtained when the accelerator was stimulated alone, it seems probable that the vagus may have been still sufficiently irritable to have caused a

weakening of the beat and thus obscured the augmenting effect of the accelerators. The explanation assumes that the augmentor and accelerator fibres are distinct and that the minimal vagus action antagonized only the effect of the augmentors. An example of this effect is given in Fig. 4. The stimulations used were as a rule of short

Fig. 4. To show acceleration without augmentation. Only the contractions of the auricle are shown. The time in intervals of two seconds and the duration of the stimulus are indicated on the two lower lines.

duration, 10 to 30 seconds, the stimulus being removed as soon as the heart began to show a marked effect. The latent period, difficult to determine with exactness, was shorter the stronger the stimulus. For example, in Exp. 15 the latent period with a stimulus of 50 was 7 seconds, with a stimulus of 20 it was 13 seconds, and with a stimulus of 10 about 35 seconds. When the stimulus was strong, or was continued for a long time, evidences of fatigue were noticeable, especially toward the end of the experiment. Fig. 5 gives an instance

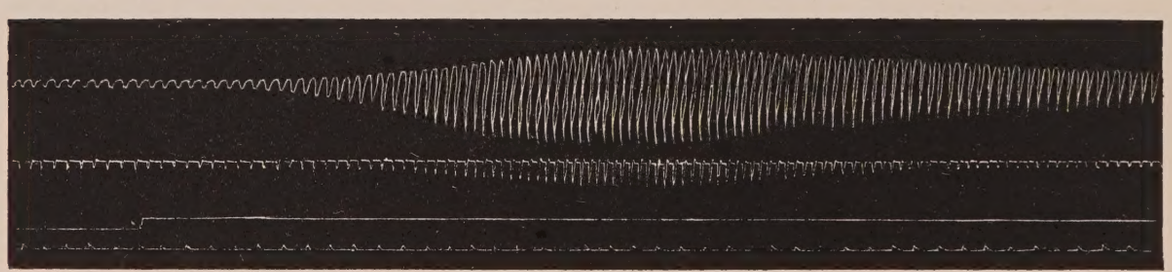

Fig. 5. To show that the acceleration and augmentation produced by a strong stimulus (100) may disappear when the stimulus is maintained for a long period. This record was obtained at the end of an experiment after the heart had been subjected to a number of stimulations and injections. The bottom line gives the time in seconds; the line above indicates the duration of the stimulation.

in which with a long continued stimulus the acceleration and augmentation passed off completely before the end of the stimulation. This stimulation was made after the heart had been isolated for some hours and had been frequently stimulated under varying conditions. In other cases it was noticed that after the acceleration produced by the stimulus had passed off there was for a time a marked diminution in rate below the normal, a phenomenon which, as stated below, was usually

apparent after injection of solutions containing an excess of calcium chloride.

Effect on the action of the accelerator nerve of variations in the percentage of calcium chloride in the circulating liquid. In regard to vagus inhibition it has been shown that within certain limits an increase in the potassium contents of the circulating liquid is accompanied by an increased sensitiveness to vagus inhibition and, on the other hand, a complete removal of the potassium salts is followed by a marked diminution in or loss of the phenomenon of vagus inhibition[1]. The chief object of our experiments was to determine whether a similar relationship can be established between the calcium contents and the action of the accelerator nerves. For this purpose numerous experiments were made in which the calcium chloride in the circulating liquid was varied from 0·006 p.c. to 0·092 p.c. The results of these experiments were somewhat inconstant; that is to say, in some cases small changes in the calcium contents made no difference in the effects of stimulation, while in other cases a distinct and constant result was obtained. In general it was found that an increase in the calcium chloride from 0·006 p.c. to 0·046 p.c. favoured the action of the nerve, in some cases in a very marked way. An increase to or above 0·069 p.c., on the contrary, had an opposite effect. When the calcium chloride was removed entirely the heart soon ceased to beat, or beat so feebly and irregularly that no satisfactory observations could be made upon its rate or strength. With a content of 0·006 p.c. of calcium chloride the heart beat feebly and the ventricle in some cases exhibited irregular contractions, oftentimes an alternating beat. Under such conditions stimulation of the accelerators with weak stimuli had no effect, or at best caused a slight augmentation. Stronger stimuli caused a greater augmentation and, perhaps, a short lasting acceleration during which the ventricular beats became regular. An increase in the percentage of calcium chloride from this point was accompanied always by a marked improvement in the force of the heart-beat, increasing as the calcium was increased. The effect upon the rate was variable, so far at least as the permanent effect was concerned. In some cases it remained unchanged, in others the rate was quickened, while in others still there was a slower beat, particularly when the percentage of calcium chloride exceeded 0·05 p.c. When the percentage of calcium chloride reached normal, that is to say 0·023 p.c., weak stimulation of the accelerators which had previously been ineffective now, as a

[1] Howell, *loc. cit.* 1906.

rule, caused marked augmentation or augmentation and acceleration. An increase to 0·046 or 0·069 p.c. might result in a still more marked effect from stimulation of the accelerators, but when this limit was passed and the heart was beating with abnormal force on account of the high calcium content, stimulation of the accelerators ceased to have an effect. The condition seems to be parallel to that observed in the relation between potassium and vagus inhibition.

Increase in the potassium contents favours the vagus inhibition, until the potassium percentage is so high as itself to cause a marked slowing and weakening of the beats; at this point stimulation of the vagus ceases to affect the heart. The following specific instances may be quoted to show this favourable action of the increase in calcium upon the effect of stimulating the accelerators. The heart was allowed to run on each percentage of calcium chloride for at least five minutes before stimulating the accelerator nerves.

	Calcium percentage	Stimulus	Acceleration	Augmentation (Ventricle)
Exp. 11	0·046	80	79·5 to 102	14 to 24 mm.
	0·011	80	None (90—90)	7 to 13 mm.
	0·006	80	None (114—114)	2 to 5 mm.
Exp. 12	0·023	20	84—108	9 to 21 mm.
	0·011	20—30	None (79·5—79·5)	None (6 mm.)
	0·006	40	None (90—93)	None (2 mm.)
	0·023	40	87 to 138	8 to 21 mm.
Exp. 14	0·011	20	84 to 108	10 to 16 mm.
	0·006	20	None	None (ventricle irregular)
	0·023	20	96 to 114	7 to 12 mm.
Exp. 15	0·023	50	90 to 96	3 to 10 mm.
	0·046	50	94 to 106	4 to 12·5 mm.
	0·046	20	None (87)	5 to 11 mm.
	0·023	20	None (90)	4 to 8 mm.
	0·069	20	88 to 108	5 to 10 mm.
	0·023	20	78 to 96	5 to 8 mm.
	0·006	20	None (64)	None
	0·023	20	66 to 90	6 to 9
Exp. 9	0·046	20	102 to 117	2 to 5 mm.
	0·023	20 to 100	None (99)	None
	0·046	10	60 to 72	3 to 5
	0·046	60	54 to 78	2½ to 6

A record from one of these experiments is given in Fig. 6 A and B. It shows more clearly perhaps than the figures in the tables the striking difference, on stimulation of the accelerators, between a heart fed upon

a subnormal amount of calcium salt and one supplied with a moderate excess of this important constituent.

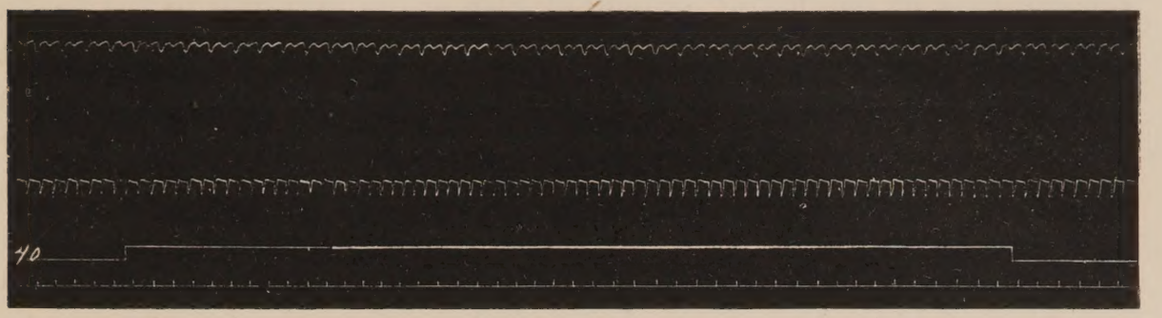

Fig. 6 A. To show the negative result of stimulating the accelerator nerve (40) after the heart had been beating for 15 mins. on a Ringer's mixture containing 0·006 per cent. of calcium chloride. The upper curve gives the ventricular contractions; the lower one the auricular contractions.

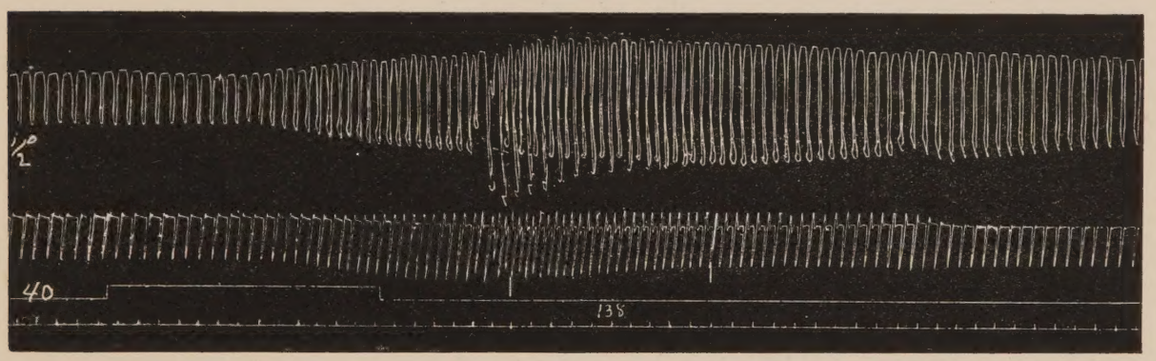

Fig. 6 B. To show the positive effect of the same stimulus (40) applied to the accelerator nerve 5 mins. after changing from the Ringer's mixture containing 0·006 per cent. of calcium chloride to one containing 0·023 per cent.

Permanent effect of variations in the calcium chloride upon the heart-rate. The experiments referred to in the preceding paragraph were practically uniform in showing that an increase in the percentage of calcium chloride in the circulating liquid is accompanied by a permanent increase in the force and amplitude of the heart-beat. This observation has been recorded, indeed, by several observers for the mammalian heart[1]. The permanent effect of this increase in calcium contents upon the rate of beat of the heart was less constant. As our experiments were made there was some difficulty in keeping the temperature absolutely constant, and this variation was a disturbing factor. While the flasks containing the circulating liquids were all kept in the same

[1] E. Gross. *Arch. f. d. ges. Physiol.* xcix. pp. 264—322. 1903. O. Langendorff and W. Hueck. *Ibid.* xcvi. pp. 473—485. 1903.

warm bath, there was frequently a difference of half a degree to a degree Centigrade which obscured the results following a change in the calcium concentration. When this factor was eliminated, however, and the temperature of the liquid, measured just at its entrance to the heart, remained uniform the results were still variable. Doubling the amount of calcium sometimes left the rate unchanged, sometimes caused a permanent increase in rate, and in some cases caused a slightly lower rate of beat. In general, however, it was found that a marked decrease in calcium, from 0·023 to 0·006 p.c., was followed by a more rapid though feebler beat, while a large increase, from 0·023 to 0·069 or 0·092 p.c., gave a more powerful but slower beat. In this latter case, moreover, it frequently happened that a partial block was established between auricles and ventricles, the rate of the latter falling to one-half, so that there was a two to one rhythm. In order to determine the initial effect of an increase in the calcium salts and to determine how far this effect corresponded with the result of stimulating the accelerator nerves, a series of experiments was made in which the calcium percentage was raised suddenly by injecting strong solutions directly into the heart.

Effect of injections of calcium chloride. In these experiments a warm solution of Ringer's mixture containing an excess of calcium chloride, 0·2 to 1·2 p.c., was allowed to run into the heart from a burette, while the heart was beating on a constant supply of the standard mixture containing 0·023 p.c. of calcium chloride. Five to twenty-five cubic centimetres of the strong solution were forced in at each injection. The effect of these injections was in every case to cause a marked increase in the amplitude of the beats of both auricles and ventricles. The beats increased gradually in force to a maximum and then declined to

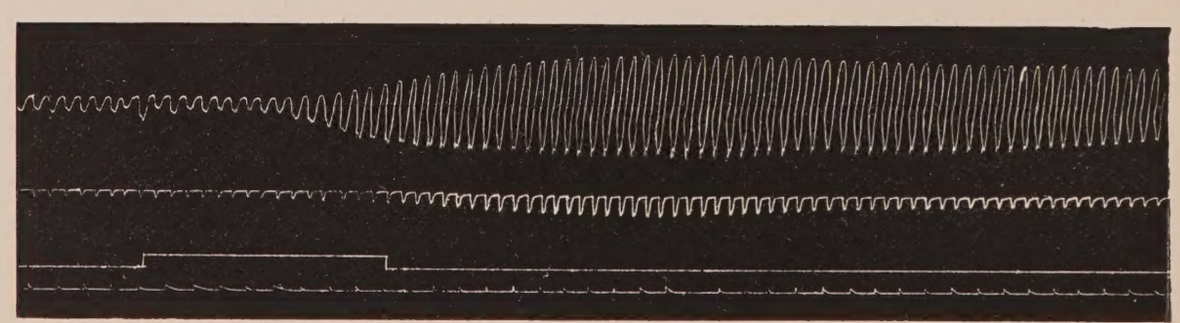

Fig. 7. To show the augmentation and acceleration caused by an injection of an excess of calcium chloride into the circulating liquid. 25 c.c. of warm Ringer's solution containing 1 per cent. of calcium chloride were injected during the interval marked by the break in the line (second line from bottom).

their normal size just as in the case of stimulation of the accelerators. An illustration of this effect is given in Fig. 7, representing the result of an injection of 25 c.c. of a Ringer's mixture containing 1 p.c. of calcium chloride. The effect of these injections upon the rate of beat was, however, less constant. In some cases, particularly when the increase in calcium was relatively small, there was no change at all in rate; the heart-beats showed simply an augmentation. In other cases, particularly with strong doses, there was a distinct although not marked acceleration which might be followed, as after stimulation of the accelerator nerves, by a fall in the rate below the normal. In the experiment chosen for illustration, Fig. 7, it will be noted that there is an increase in rate from 108 to 114 beats per minute. The effect upon the rate was most marked when the heart had been previously submitted to the action of an excess of potassium chloride, so as to cause a marked slowing or complete inhibition. In such cases an injection of calcium chloride caused the heart to beat, if it was previously completely inhibited, and the beats, at first slow, increased to a very rapid rate and then again fell off to a state perhaps of complete inhibition. If the increase in calcium contents was not too large and the injection was not made too suddenly the contractions of both auricles and ventricles increased uniformly in size and perhaps in rate without any indication of irregularity in rhythm. With strong doses, however, the ventricle especially might show a temporary arhythm and irregularity, as from overstimulation; an effect which, it may be noted, was not infrequently observed also during stimulation of the accelerator nerves. In fact the effect upon the heart of a sudden temporary increase in the calcium contents of the circulating liquid bore a striking resemblance to the effect of stimulating the accelerators. Frequently one could not tell from the records alone whether a given effect had been obtained by stimulating the accelerators or by injecting calcium solutions; the most marked difference between the two actions was that stimulation of the nerve as a rule caused a more marked acceleration.

Effect of injecting solutions of sodium or potassium chloride. Experiments were made similar to those just described with the exception that the solutions injected contained an excess of either sodium or potassium chloride. Regarding the latter substance the effect was that usually described. The size and rate of the beats were diminished, and, if the concentration was sufficient, the heart was brought to rest in a state of potassium inhibition. From this condition it soon returned

to a normal beat when the standard Ringer's mixture was allowed to flow through it. When the sodium chloride was injected the first effect was a diminution in size of the heart-beats, affecting both auricles and ventricles, without any change in rate. If the dose was small (10 c.c. of solution containing 2 p.c. of sodium chloride) this effect was very temporary; the heart soon returned to its normal beat. With stronger solutions (10 to 15 c.c. of a 5 p.c. solution) the weakening of the beat was more pronounced, so that a scarcely visible record was obtained. From this condition the heart recovered somewhat slowly and then for a time gave a series of beats larger than normal, but at a somewhat slower rate.

The effect of variations in the concentration of the potassium chloride upon the action of the accelerator nerves. A few experiments were made to determine what effect, if any, is exerted upon the action of the accelerator nerves by moderate changes in the concentration of the potassium chloride of the Ringer's mixture. The results of these experiments were not so definite as in the case of the similar ones made with calcium chloride. In the records obtained the only constant result observed was that as the percentage of potassium was increased the rate and especially the size of the beats were diminished, and upon stimulating the accelerators the augmentation obtained was progressively less marked. As regards the effect upon acceleration it was found that when the amount of potassium was sufficient to give a marked slowing of the beat the percentage acceleration was increased, although the absolute rate was less than upon the standard mixture. The nature of the results obtained may be seen from the following summary of two experiments.

	Percentage of KCl	Acceleration during stimulation	Augmentation of ventricular contraction
Exp. 17	0·025	73·5 to 108	9 to 12 mm.
	0·037	69 to 108	6 to 10 mm.
	0·050	64·5 to 108	5 to 8·5 mm.
	0·075	46·5 to 90	4 to 6 mm.
	0·125	Complete inhibition	
	0·025	49·5 to 126	7 to 11 mm.
	0·013	51 to 96	5 to 9·5 mm.
	0·000	60 to 96	9 to 13 mm.
	0·025	60 to 84	2 to 5 mm.
Exp. 18	0·025	None (102)	11 to 16 mm.
	0·000	None (84 to 87)	13 to 16 mm.
	0·025	None (105)	9 to 13 mm.
	0·050	None (93)	7½ to 14 mm.
	0·075	66 to 90	4 to 10 mm.
	0·100	15 to 45	2 to 11 mm.

Stimulation of the accelerator nerve during complete potassium inhibition. When the percentage of potassium chloride in the circulating mixture was increased to 0·100 or 0·125 the heart was completely inhibited. So far as could be determined by careful inspection all parts of the heart were completely at rest. Stimulation of the accelerator nerve with strong stimuli under these conditions called forth a short or long series of beats which showed the usual increase in size and rate to a maximum and a subsequent decline to zero. The duration of this series of contractions and the maximum rate reached depended on the depth of the potassium inhibition and the strength of the stimulus. Fig. 8 gives a typical instance of this effect. The heart had been

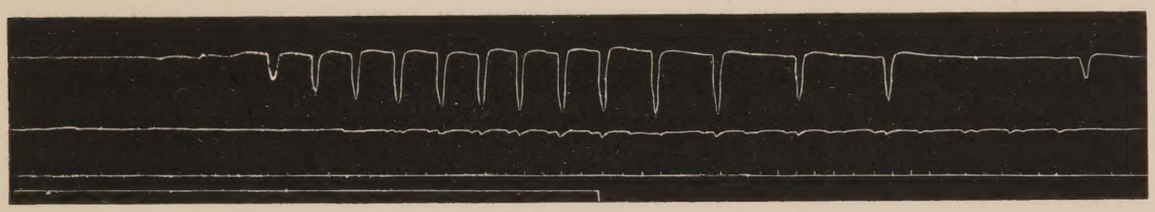

Fig. 8. To show the series of beats caused by stimulation of the accelerator nerve while the heart was in a condition of complete potassium inhibition produced by a Ringer's mixture containing 0·1 per cent. of potassium chloride. Upper record shows the contractions of the right ventricle; the lower one the contractions of the left auricle. The duration of the stimulus is indicated by the break in the bottom line. Time in seconds.

stopped with a Ringer's mixture containing 0·100 p.c. of potassium chloride. A very strong stimulus was thrown into the nerve. The first effect, after a long latent period, was the appearance of feeble but regular contractions of the auricles, too weak to be registered. These increased in strength and finally the ventricle began to beat with increasing force. After the cessation of the stimulus, the beats rapidly decreased in force and rate. In another instance the heart was stopped on a mixture containing 0·125 p.c. of potassium chloride. A strong stimulus (300) applied for 46 seconds caused a series of feeble beats lasting 4 minutes. The heart was then given a mixture containing only 0·075 p.c. of potassium chloride. It failed to beat on this mixture, but evidently the intensity of the inhibition was diminished, since the same stimulus applied to the nerve for 25 seconds aroused a series of forcible beats which continued for over 3 minutes. There has been some question whether the accelerator nerve, like a true motor nerve, is capable of stimulating the quiescent ventricle, giving rise therefore to the production of a so-called inner stimulus, or whether its activity

is confined to regulating the rate and strength of the spontaneously beating heart. The apparent cases in which after muscarin or heat standstill the heart has been made to beat during stimulation of the accelerator nerves have been explained away by Gaskell[1] on the supposition that the sinus end of the heart was still beating and that stimulation of the accelerators simply strengthened and established this rhythm by removing the block. Hering[2] reports that he has made the auricle beat by stimulation of the accelerator after it had been brought to rest by potassium, stoppage of circulation, etc. In all such cases however it seems that the ventricle was either beating or fibrillating. In our cases of potassium inhibition no contraction of any kind could be detected in either the auricles or the ventricles, and it would seem to be entirely gratuitous to assume that spontaneous contractions too small to be visible were still taking place. One might with equal right assume that in vagus inhibition also the rhythm is never suspended, but that the contractions are simply rendered invisible. It seems to the authors that there can be no reasonable doubt of the fact that in these experiments the heart during the potassium inhibition was absolutely at rest, the automatic rhythm was entirely suspended, and from this condition of rest the heart was stimulated to give a series of beats by excitation of the accelerator nerves. A very similar effect may be produced upon the heart while in a condition of potassium inhibition by injecting a mixture containing an excess of calcium chloride. Whatever may be the nature of the initial process leading to a normal heart-beat it is one that may be aroused by nerve impulses sent through the accelerator nerve fibres or by increasing the amount of calcium compounds in the circulation liquid. The similarity under these conditions between the effect of stimulating the accelerator fibres and of increasing the amount of calcium in the circulating liquid is striking.

Some observations on the effect of stimulating the vagus nerve in the isolated heart. It was found in our experiments that the inhibitory mechanism of the heart lost its irritability much sooner than the accelerator apparatus. On this account comparative experiments upon the effect on inhibition of variations in the concentration of the salts were difficult to interpret. So far as our results went they supported

[1] W. H. Gaskell. *Text-book of Physiology*, edited by Schäfer, II. p. 217. 1900.

[2] H. E. Hering. "Einiges über die Ursprungsreize des Säugethierherzens und ihre Beziehung zum Accelerans." *Zentralb. f. Physiol.* XIX. pp. 129—136. 1905.

those described by one of us from experiments upon the cold-blooded heart, that is, an increase in the potassium salts favoured the action of the inhibitory fibres, while a decrease or total removal of this constituent lessened the effect of stimulating these fibres. On the other hand, variations in the calcium concentration exerted no perceptible effect on stimulation of the vagus. Thus in one experiment complete inhibition was obtained with current strengths of 15 when the heart was fed with Ringer's mixtures whose percentage of calcium chloride varied from 0·023 to 0·092; and with a stimulus of 10 a weakening of the force of the beat was observed. When the potassium was reduced to zero, the calcium chloride being normal (0·023) no inhibition could be obtained with current strengths of 30. On changing the solution to one containing 0·025 p.c. of potassium chloride a stimulus of 30 caused inhibition of the auricles. The recorded effects of vagus stimulation showed some interesting variations. Usually as the strength of the stimulus was reduced the effect upon the heart-rate fell out, the only result of the stimulation being a marked reduction in the force of the beat either of both chambers or of the auricles alone. In some cases vagus stimulation of a certain strength caused a reduction in the force of the auricular beat almost to the point of invisibility without exercising any influence upon the force of the ventricular beat, while in other cases the ventricle ceased to beat some time before the auricles. This last result was interesting as indicating that the vagus nerve may have a direct influence upon the ventricles, a point that has been rendered doubtful for the mammalian heart by recent work[1].

Such an effect as the one last mentioned may of course be explained either by supposing that the vagus affected the ventricles more promptly than the auricles, or by assuming that as the force of the auricular beats gradually declined the auricular impulse became too feeble to stimulate the ventricles, and the contractions of these chambers stopped therefore before the auricles were completely inhibited. In one interesting case, however, reproduced in Fig 9, the ventricular beats were slowed and weakened, while the auricular beats were slowed and augmented. Such a result as this can scarcely be understood except upon the view that the vagus fibres end in the ventricular muscle and are capable of affecting its contractions directly. In all of these experiments, however, as in those described by other observers, the general fact was clear that the influence of the vagus upon the auricles is more pronounced than

[1] Erlanger and Hirschfelder. *Amer. Journ. of Physiol.* xv. p. 153. 1906.

upon the ventricles. Another interesting point observed in several cases was that after circulating a mixture containing no potassium chloride, a sudden change to the standard mixture with potassium chloride at 0·023 p.c. was followed by a complete inhibition of the heart-beat for a number of seconds. From this condition the heart soon recovered and beat with its accustomed regularity. It would seem that after reducing the amount of diffusible potassium salts in the heart tissues a sudden change to the normal amount may result in a temporary potassium inhibition.

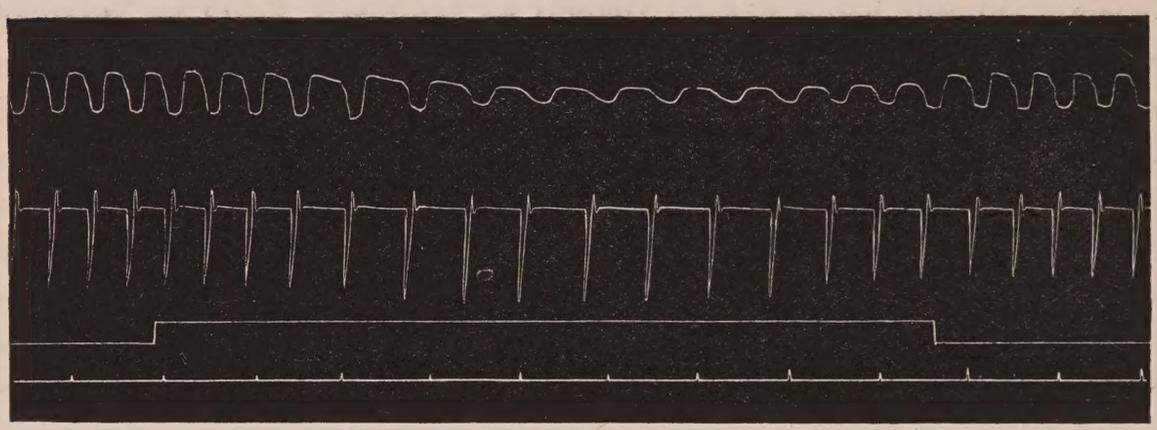

Fig. 9. An instance of vagus stimulation in which, while both auricle and ventricle were slowed, the contractions of the ventricle (upper curve, right ventricle) were weakened, while those of the auricle (lower curve, left auricle) were augmented. The result indicates that the vagus fibres have some direct control over the ventricle.

Theoretical considerations. As stated at the beginning of the paper, the central idea of the investigation was to determine the relation between the concentration of the calcium chloride in the circulatory medium and the action of the accelerator nerve. The results show that the action of this nerve is distinctly favoured by an increase in the calcium constituent within limits not too extreme. Moreover a sudden temporary increase in the calcium contents produced by injecting strong solutions of calcium chloride gives effects which closely simulate those produced by stimulating the accelerator nerve. A resemblance between the two effects was observed not only in the general character of the records, but also in some of the subsidiary and occasional features. Thus in one experiment stimulation of the accelerators, contrary to the rule, caused first a slight diminution in the size of the beat before the regularly increasing augmentation set in. Injection of calcium solutions gave a record in which the same peculiarity was shown. The after-effects of the two actions were also alike in that frequently while the

effect of the stimulus or the injection was passing off there was a distinct slowing of the rate of beat for a short time. Strong stimulation of the nerve, like large injections of the calcium solution, caused occasionally a passing irregularity in the ventricular beat before it settled down to a regularly augmented rhythm. It will be remembered also that when the heart was thrown into complete diastole by the action of an excess of potassium chloride a series of beats might be aroused either by strong stimulation of the accelerator nerve or by injecting an appropriate amount of calcium chloride into the circulating liquid. These resemblances give some colour to the view that the action of the augmentor nerve impulses consists essentially in liberating calcium in diffusible form within the substance of the heart, in the same way that the vagus impulses may be supposed to liberate diffusible potassium. One of us has elsewhere expressed the opinion[1] that the spontaneous beat of the heart may be explained best in the light of our present knowledge on the hypothesis that the energy-yielding material in the heart is combined with potassium or potassium salts to form a stable and slightly dissociable compound, and that under normal conditions the calcium salts in the tissue lymph react with the dissociated portion of this material to form a calcium compound which is unstable and which breaks down spontaneously when a certain amount of calcium is combined. This latter dissociation may be regarded as the initial step in the heart contraction, and it is followed by an oxidation, or hydrolysis and oxidation, of the organic portion of the compound, whereby the energy of the contraction is furnished. The force of the contraction will depend ultimately upon the amount of the unstable calcium compound which is formed, and we may assume that this amount will vary with the relative proportions of calcium and potassium salts present in the tissue lymph. An excess of the former will lead to an augmented and perhaps an accelerated contraction, while an increase in the potassium will result in a diminished contraction, or, if the concentration is sufficient, in complete inhibition. If we suppose that the vagus fibres end in a portion of the muscular substance rich in potassium and that the effect of its impulses is to liberate an excess of this material by causing dissociation, while the accelerator fibres end in a calcium containing substance and that the effect of its impulses is to increase the number of calcium ions, then the opposite actions of these two nerves and the similarities shown to the effects of artificially increasing

[1] Howell, *Journ. Amer. Med. Assoc.* Nos. 22, 23. 1906.

the concentration of the potassium and calcium salts respectively find a plausible explanation. So far as the accelerator nerve fibres are concerned it is frequently suggested that two varieties exist[1], the accelerator fibres proper which increase the rate, and the augmentor fibres which increase the size of the beat. The observations recorded in this paper are in accord with this view to the extent that augmentation was obtained frequently under certain conditions without an acceleration of rate, and in one experiment an acceleration occurred without augmentation. The effect upon the heart of increasing the concentration of the calcium chloride in the circulating liquid was expressed mainly as an augmentation of the beat, the effect upon rate was less marked and less constant, and not infrequently was absent altogether. The hypothesis outlined above applies especially therefore to the augmenting action of the accelerator nerve or to the action of the augmentor fibres. To make the hypothesis complete it would be necessary to assume further that the accelerator fibres proper, after the manner of the motor fibres to ordinary muscle, hasten prematurely the decomposition of the unstable calcium compound of the energy yielding substance, just as occurs when an electric shock is sent into the heart as an extra stimulus during the early part of diastole. In accordance with this form of the hypothesis acceleration might occur without augmentation or even with a weakened beat, a result that has been observed a number of times. On the other hand, the action of the augmentor fibres if stimulated alone or chiefly might cause an augmentation of the contractions with little or no change in rate.

Summary.

The paper reports a series of experiments made upon the isolated heart of the cat. In these experiments an effort was made to study the effect of stimulating the accelerator and the inhibitory nerves in relation to definite changes in the concentration of the several inorganic salts contained in the circulating liquid. Especial attention was paid to the relation between the concentration in calcium salts and the action of the accelerator nerve. The following conclusions were reached.

1. The mammalian heart kept beating upon an artificial circulation of Ringer's solution, as modified by Locke, shows the effect of stimulation of the accelerator nerves for many hours. In the animal used

[1] R. Hunt. *Amer. Journ. of Physiol.* II. pp. 395—470. 1899.

(cat) the effect of stimulating the left accelerator was an augmentation as well as acceleration. With strong stimuli the increase in the force of the heart-beat was remarkable. With weak stimuli augmentation alone as a rule was obtained.

2. An increase in the percentage of calcium chloride in the circulating liquid from 0·006 to 0·092 p.c. caused a corresponding increase in the force of the auricular and ventricular beats. The permanent effect of changes in this constituent on the heart-rate was not constant. In general a very marked reduction in the calcium chloride (0·006 to 0·011 p.c.) was followed by a more rapid as well as a more feeble beat. While a large increase (0·069 to 0·092 p.c.) was followed by a much stronger but slower beat. Changes within these limits, while altering the force, might have no permanent effect upon the rate of the beat.

3. A sudden increase in the concentration of the calcium chloride, caused by injecting into the circulating mixture strong solutions of the salt, gave an effect similar to that caused by stimulation of the accelerator nerve. Both augmentation and acceleration might follow the injection, but the augmentation was the most pronounced and constant result. With small doses augmentation only was obtained.

4. A sudden increase in the concentration of the sodium chloride diminished the force of the beat although subsequently a slight augmentation might result together with a reduction in the rate. Increase in the potassium chloride caused a weakening and slowing of the beat which terminated finally in potassium inhibition at a concentration of 0·100 to 0·125 p.c.

5. Within certain limits the action of the accelerator nerve was favoured by an increase in the concentration of the calcium chloride. At concentrations of 0·006 p.c. the effect of stimulating the nerve was uncertain and might be entirely negative. An increase beyond this point to 0·046 p.c. restored the action of the accelerator and rendered the heart more sensitive to its influence. At concentrations of 0·069 to 0·092 p.c. when the calcium effect upon the force of the beat was strongly marked, the accelerator might again fail to have an action on the heart.

6. When the heart was brought completely to rest by an excess of potassium chloride, strong stimulation of the accelerator caused a series

of beats, the force and duration of which varied with the intensity of the potassium inhibition and the strength of the stimulus.

7. Variations in the concentration of the potassium chloride had no distinct influence upon the action of the accelerator, except that as the force of the beat was diminished by the potassium the augmenting effect of the accelerator was correspondingly reduced.

8. Variations in the concentration of the calcium chloride seemed to have no distinct effect upon the inhibitory influence of the vagus nerve.

9. Some evidence is furnished to show that the inhibitory influence of the vagus may be exerted upon the ventricles directly as well as indirectly through the auricles.

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